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OF IXODIDAE OFF OF ANIMALS AND GAMASIDAE FROM NESTING MATERIAL

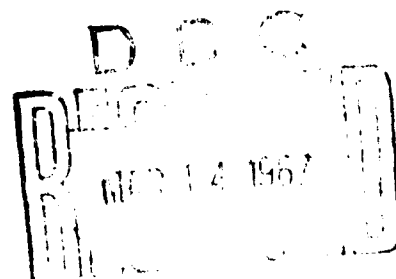
AD 648113
767-61208

Translation No. 1697

May 1966

U. S. ARMY
BIOLOGICAL LABORATORIES
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THERMO-ELECTOR WITH WATER JACKET HEATING FOR TAKING THE LARVAE AND NYMPHS OF IXODIDAE OFF OF ANIMALS AND GAMASIDAE FROM NESTING MATERIAL

[Following is the translation of an article by V. A. Merinov, Department of Entomology, Institute of Medical Parasitology and Tropical Medicine, USSR Ministry of Public Health, published in the Russian-language periodical Meditinskaya Parazitologiya i Parazitarnyye Bolezni (Medical Parasitology and Parasitic Diseases), Vol 42, 1964 No.5, pages 577--582. It was submitted on 19 Jun 1962. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The laboratory methods for collecting engorged Ixodidae larvae and nymphs or feeding them in large batches are laborious and detailed. Following "free feeding" it is necessary to recover the engorged larvae and nymphs either from the water (if the container with the animals is placed over a cuvette with water), or from the sawdust, excrement, and food remnants (when the animals are kept in glass or metal cages). N.G. Olsufyef's method (1941) for the collection larvae and nymphs which drop off an animal, and which uses the help of a perforated cage enclosed in a gauze bag, makes the operation easier, but preserves the manual collecting of the larvae and nymphs from filter paper, a small tray or from the walls of the bag. Moreover, all these methods do not guarantee safety when operating with infected material. Finally, with manual collection some of the larvae may be harmed.

The proposed TEV-3 thermo-elector with water jacket heating (priority as of 3 Oct 1959, Patent Certificate No 133191) eases considerably the collection of pre-imago phases of Ixodidae and may also be used for collecting Gamasidae from nests. Little collars to prevent the animal from scraping off the ticks remain necessary. The TEV-3 elector ensures safety when working with contaminated ticks; the path from the animal to the test tube in which the larvae and nymphs are taken away is completely isolated; they reach the test tube without contamination and moisture. The thermo-elector may also be used in field investigations for collecting ticks from small natural sources of nourishment, especially if it is important to keep the latter alive. If there is no electrical network present it is possible to use an electric heater, fed from a vehicle battery, or, having replaced several parts of integral glass with tin and altered the design of a tub, to adapt a tub for heating with a kerosene lamp.

The thermo-elector (figures 1, 2 and 3) consists of four main parts; a three-surfaced chamber(1), tub (2) with a tray for water (3), electric heater (4) and thermo-relay (contact thermometer 5) with a magnetic relay (6) for regulating the temperature of the water. The three-sided chamber and the tub with tray are made from organic sheet glass with a thickness of 3 mm and installed as one piece. The 0.4--0.5 liter capacity tub is constructed in such a way that when it is filled with water the flat sides of the chamber are washed off and the closer it is to the apical corner of the chamber then the less amount of water there is for heating these sides. This is achieved by the triangular form of the arms of the tub, encompassing the

chamber from the sides. With such a construction the highest temperature is created at the near surface of the chamber, and the temperature of the lateral surfaces gradually drops toward the apical verge in connection with the decreased volume of water and the greater heat transfer.

In the lower part of the chamber, a tray is placed to form the bottom platform of the chamber (7) and also a secondary bottom (8). The tray is connected with the tub and the level of water is regulated with a screw (9). When the regulator is open the water displaces the air and the tray fills to capacity. By establishing the water level in the tray, it is possible to regulate the temperature of the bottom platform. Since engorged larvae possess negative geotaxis, the bottom platform is mounted at an angle of 18--20°. If food remnants and feces fall through the edge of the filter, then, by rolling below to the base of the platform, they do not block the outlet tube. In the upper verge of the chamber the bottom platform converts into a glass outlet tube (10) with a length of 50 mm and a diameter of 9mm, and found at the same angle.

Somewhat higher (over the bottom platform) the inner surface of the sides of the chamber is framed with a strip (11), directed at an angle of 10--12°. This forms guiding channels with the plane of the lateral surfaces. In the apical corner of the chamber the lower surface of the channel interlocks with the upper surface of the outlet tube.

In this manner, the larvae and nymphs, urged on by an unfavorable temperature and a high moisture deficiency, crawl into the outlet tube either by way of the bottom platform or by way of the guiding channels. For those larvae and nymphs which nevertheless pass through the barrier strip, and mainly for those of these which crawl onto the sides of the chamber directly from the screen, a supplementary barrier is mounted in the upper part of the chamber. This barrier (12) is mounted at an angle of 20--22° and has deeper guiding channels. These channels lead to an upper outlet tube.

Test tubes, moistened according to the method of Shurenkova--Nelzina, are placed on the upper and lower tubes. In the middle part of the chamber a triangular wire frame (13) is let down on small supports. The frame is covered with a metallic, porous screen (up to 10--15 mm). The animal with the collar is placed on the screen. Wild or especially infected animals are placed in a netted container, for which this screen serves as the bottom. In order that the animal does not contaminate the bottom platform a filter (14) is suspended under the screen. It is a multilayer filter paper folded into a triangle. The creases which are formed when folding the filter paper (they should be as small as possible) are stitched with soft wire or strong thread. The prepared triangle is carefully (not strongly) folded through the bisecting lines of the angles in such a way that a concave surface is formed in the central part of the filter. The filter is suspended on wires under the screen at a distance of 8--10 mm from it so that its center is located under the center of the screen.

The space between the sides of the chamber and the edges of the filter should not be great: In the opposite case food remnants and excrement will fall through and contaminate the bottom platform, making movement for the larvae and nymphs more difficult. For nymphs this space can be 6--7 mm, and for larvae -- 3 mm. Adjustment and attachment of the filter to the screen is performed outside of the chamber. Then assayer's tongs are used

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to lower the screen with the filter onto the supports in the central part of the chamber. For animals which are placed in the eclector without a cage (mice, guinea pigs) a drinking bowl (15) is mounted over the screen on the rear side.

The upper part of the chamber is ringed inside with an inclined flange (16). Glycerin or water is poured into the groove formed in this manner (or the walls of the groove and the internal surfaces of the chamber of this groove are smeared with vaseline) in order to prevent the ticks from penetrating the barrier:

A small and a large model (see figures 2 and 3) were constructed and tested. The small model is intended for the collection of larvae from white mice and other small animals. It can be recommended for the carrying out of experimental virological investigations during which the biological tests are set up on these animals (see figure 1). The large model is intended for larger animals, for example guinea pigs, on which biological tests are set up when working with certain rickettsial diseases.

In the large model the tub is made from thicker organic glass (5 mm), and its capacity is increased up to 2.5 liters in connection with the greater heating area of the rear and side surfaces. For decreasing the path of movement for the larvae and nymphs which are dropping off along the bottom platform up to the outlet tube, the prismatic three-sided chamber of the small model is replaced by a truncated-pyramidal three-sided chamber, tapering downward and thus reducing the size of the bottom platform. The dimensions of the electric heater are increased.

Since a guinea pig produces much fluid excrement, the filter is prepared from numerous sheets of paper. In order to prevent the running off of excrement along the walls of the chamber onto the bottom platform (especially if it is taken into consideration that a pig with a collar is sitting, "resting" with the rear paws in a corner of the chamber), wire railing-arresting devices with a height of 50--55 mm are fastened to the screen at a certain distance from its edge (15--20 mm), or a sectional three-sided try square made out of organic glass with a depth of 55-70 mm is enclosed. The animal sits in this triangle-arresting device. In order that less crumbs of food fall onto the bottom platform, plate-rims made of organic glass, and sloping toward the center of the chamber, are put on the screen along the sides of the chamber and close to it.

We will present the data from several tests when using the TEV-3. In all the tests the animals were placed in the eclector before the larvae and nymphs began to start to drop off, that is in two days after the release of the larvae or 4 days after the release of the nymphs. The larvae were reared on white mice. For the white mice pieces of white bread weighing 15--20 grams were deposited on the screen daily and a pipette was used to pour water into the water bowl. The mice remained in the eclector for 4--7 days (a period which at the increased temperature of the chamber is sufficient for the complete engorgement of almost all the larvae). The results of the tests are presented in table 1.

For the successful recovery of ticks it is necessary to select the most favorable water temperature in the tub which would not create sharply desiccating conditions. Thus the larvae of *D. nuttalli* are driven off

with a water temperature of 42--43°, the base of the bottom platform is 39--40° and at the apex it is 26--27°. Larvae of *I. persulcatus* are driven off considerably better at a water temperature in the bath of 28--39°, at the base of the bottom platform it is 26--28°, and at the apex -- 23--24°.

The large model was used for the collection of *I. persulcatus* larvae and *D. nuttalli* and *D. marginatus* nymphs from guinea pigs experimentally infected with rickettsiae of Northern Asian tick-borne typhus. The guinea pigs were fattened with moistened white bread and beets (table 2).

An electrothermometer was used for the daily measuring of the pigs' body temperatures. Measurements were made in the socket between the shoulders following a light pressing in of the sensitive element of the electrothermometer. As we were convinced, the skin temperature of the pig in this part of the body was 2.0--2.5° lower than that obtained by rectal measurement. The sensitive unit of the electrothermometer was attached on a special plastic extension and lowered into the eclector onto the back of the animal. This method eliminates direct contact with an infected pig, and speeds up and eases the measuring of temperature.

As is obvious from the tests, the best result in driving off *D. marginatus* nymphs is obtained at a temperature of water in the tub of 50--52°, at the base of the bottom platform it is 42.5--44° and at the apex 24.5--26°. Favorable temperatures for driving off *I. persulcatus* nymphs -- water in the tub 28--29°, at the base of the bottom platform 26.5--27°, and at the apex 23--24°.

The results of the tests for driving Gamasidae ticks out of nesting material are presented in table 3.

On the bottom platform of the device we placed a small amount of substrate (rotten wood, fur, wilted grass, dirt, grain) with a humidity of 57% from a jar with a culture of *H. casalis* and Tyroglyphidae ticks. When using the small model, in the first day of the test the condensation of moisture took place in the upper part of the chamber (at the second barrier), and the driving off of ticks was slowed down. By the end of the first day the driving off had increased, and on the second day, when the material had dried up considerably, not only were *H. casalis* driven into the test tube but also Tyroglyphidae ticks. For the driving off of *H. casalis* the large model yielded the best results. (see table 3). Already in 2--3 hours the main mass of ticks of this species had been driven out of a 75 gram batch of substrate with a humidity of 53%. Good results were also obtained in driving out *O. bacoti*. The large model is convenient for driving out Gamasidae ticks, since it makes it possible to store more nesting material. Moreover, the condensation of moisture on the sides of the chamber is weaker and the driving out takes place considerably more rapidly.

[Following is the English summary which appears with the Russian article.]

The proposed thermo-eclector with water jacket heating (priority of

October 3, 1959, Patent certificate No 133191) facilitates the collection of young Ixodidae from animals and is also applicable for obtaining Gamasidae from birds' nests.

The design features of the thermo-elector ~~are such as to~~ provide for a mechanical collection of ticks. Their basic aim is to create for ticks unfavorable temperature conditions and a high degree moisture deficiency. The collection of ticks from smaller animals is realized by a miniature model, while driving the ticks off the big animals and collection of gamasides from nests is effected with the aid of a grand model. The use of a thermal relay makes it possible to select for the driving off operation optimal temperature conditions and to maintain them at a set level through out the whole period of the experiment.

In driving off the *D. nuttalli* and *D. pictus* larvae good results were achieved with water temperature in the jacket (bath) kept at 42--43° and in the bottom space - at 39--40°; *I. persulcatus* larvae -- at 28--30° and 26--28° respectively; *D. marginatus* nymphs (with the grand model) at 50--52° and 42.5--44° respectively. Optimal conditions for driving gamasides off the nests (*H. casalis*, gamasides of the starling nests) - provide for the temperature in the jacket of the grand model equalling 42--45°. For driving off with the aid of a miniature model from a fairly dry material it suffices to set the thermal relay contactor at 35--36°. The grand model is suited better for driving the gamasides off the nests.]

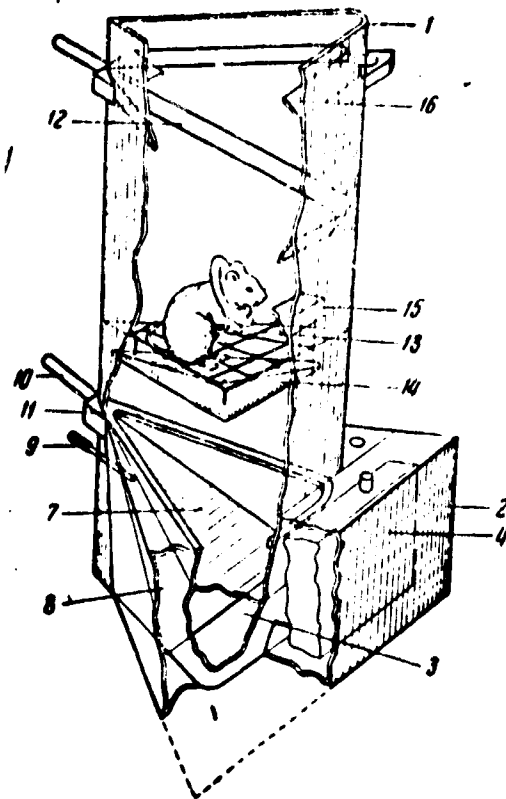


Figure 1. Schematic drawing of a model of the thermo-elector (explanation in the text).

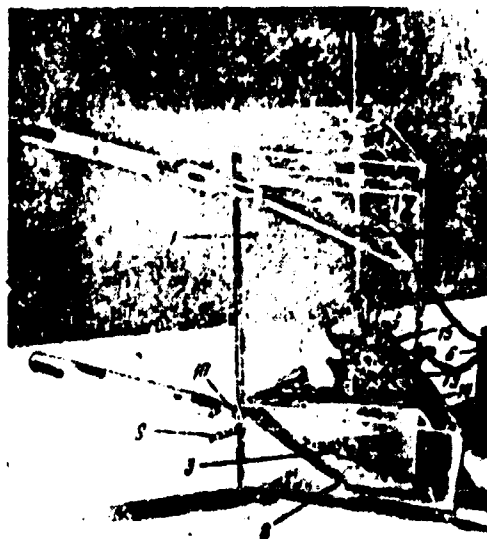


Figure 2. General view of the driving off of ticks from the animal (small model).

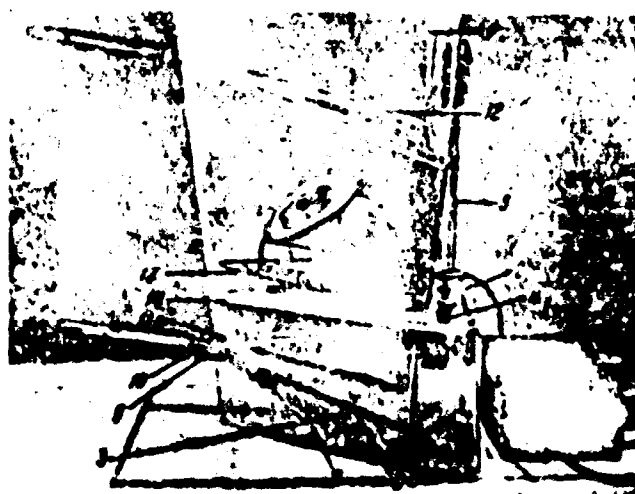


Figure 3. General view of the driving off of ticks from the animal (large model).

Table 1

Results of driving off of Ixodidae larvae, which were fed on mice, using the small model of the TEV-3.

Species of ticks	Stay in eclector (in days)	Temperature (in degrees)			Driving off (Absolute nos.)			Remained in vase-line, died (in abs. nos.)	% of driving off
		water in jacket	base of bottom platform	apex of bottom platform (4)	into lower test tube	into upper test tube	all told		
<i>D. nuttalli</i>	7	42--43	39--40	26--27	1,055	123	1,178	60	95.2
<i>I. persulcatus</i>	4	42--43	39--40	26--27	136	7	143	272	34.4
<i>I. persulcatus</i>	4	28--30	26--28	23--24	439	13	452	21	95.6
<i>D. pictus</i> (1)	5	42--43	39--40	26--27	1,890	33	1,923	217	90.0
<i>I. persulcatus</i> (2)	5	28--30	26--38	23--24	67	18	85	11	88.5
<i>D. pictus</i> (3)	3	36--37	34--35	25--26	121	21	142	23	84.0

(1) Test set up by I. V. Razumova (IMPITH).

(2) Test set up by I. V. Babenko (IMPITH).

(3) Test set up jointly with Z. M. Zhmayeva (Institute of Epidemiology and Microbiology imeni N. F. Gamaleya).

(4) To a great degree the temperature is dependent on the temperature of the surrounding air.

Table 2

Results of the collection of Ixodidae larvae and nymphs with the large model of the TEV-3

Species of ticks	Phase	Stay in collector (in days)	Temperature (in degrees)		Driving off (Absolute nos.)		Remained in vessel, died in abs. nos.)	% of driving off
			water in jacket	base of bottom platform (1)	into lower test tube	into upper test tube		
<i>I. persulcatus</i>	Z	8	28--29	26.5--27	—	—	19	82.2
<i>I. persulcatus</i>	Z	8	28--29	26.5--27	328	21	25	93.4
<i>D. nuttalli</i>	N	8	28--29	26.5--27	6	1	1	—
<i>D. marginatus</i>	N	10	42--43	39--40	34	3	60	38.1
<i>D. marginatus</i> (2)	N	8	50--52	42.5--44	84	3	9	90.7

(1) Temperature depends on the temperature of the surrounding air.

(2) Test conducted by I. V. Razumova.

Table 3

Results of driving out Gamasidae ticks with the small and large models of the TEV-3.

Model	Species of ticks	Number of days for driving out	Temperature (in degrees)			Driven out (in absolute numbers)	
			water in jacket	base of bottom platform	apex of bottom platform (2)	into lower test tube	into upper test tube
Small	H. casalis	2	45	40--41	31	140	22
	Tyroglyphidae	2	45	40--41	37	121	52
	O. bacoti (1)	3	35--36	33--34	24	861	21
Large	H. casalis	2	42--43	39.5	23.5	1,357	663
	Gamasidae from a starlings nest (3)	2	45	40--41	31	Thousands in all the mobile phases of development	

(1) Test set up jointly with A. A. Zenskaya (Institute of Epidemiology and Microbiology imeni N. F. Gamaleya).

(2) Temperature depends significantly on the temperature of the surrounding air.

(3) Field tests.